MICROTECHNICAL METHODS

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 203

W. J. G. LAND

An improved method of replacing the paraffin solvent with paraffin

In paraffin imbedding the almost universal custom when replacing a paraffin solvent with paraffin is to add from time to time small pieces of paraffin to the solvent, until at room temperature no more paraffin is dissolved. Then the container is either placed in a low temperature oven or on an oven of higher temperature and paraffin added until the new saturation point is reached. The container is then placed in an oven having a temperature one or two degrees above the melting point of the paraffin and more paraffin is added. When the last paraffin has melted the mixture is poured off and replaced with pure melted paraffin. The object in repeatedly adding small quantities of paraffin is to prevent a too rapid increase in the density of the xylol-paraffin or whatever solvent is used for paraffin.

Objects to be imbedded, being heavier than xylol, sink to the bottom. Paraffin, which is heavier than xylol, also sinks, with the result that the objects are almost immediately surrounded by a dense layer of dissolved paraffin, thereby defeating the end sought by a gradual addition of paraffin. It has repeatedly come to the notice of the writer that much of the damage to delicate plant tissues takes place during the replacement of the solvent by paraffin. Many otherwise careful workers are particularly careless at this point.

In order to prevent the object from coming into immediate contact with the dense xylol-paraffin solution, a piece of wire gauze is bent in such a manner that it forms a support 2–3 cm. above the object, and xylol sufficient to rise 1–2 mm. above the support is added. The container (a shell or bottle) is then filled with blocks

of paraffin, corked, and set aside and not disturbed until the saturation point is reached. The container is next placed on the oven until the new saturation point is reached, next in the oven, and imbedding is proceeded with in the usual manner. Even here, when dealing with delicate plants, the xylol-paraffin is poured off only to the wire gauze and the container again filled with blocks of paraffin.

By this method paraffin is slowly dissolved, and as it descends is slowly and uniformly diffused through the xylol, thus preventing, in a large measure, damage to the object by rapid changes in density. It takes longer to reach the saturation point than when solid paraffin is permitted to fall to the bottom of the container, but little plasmolysis results.

Using delicate liverworts for test objects it was found that no deformation of tissue took place, except that which can be accounted for by the excessively large coefficient of expansion (0.00027854) of paraffin. The deformation caused by paraffin in an artificial cell was found to be exactly the same as is always present in plant cells when the paraffin is quickly cooled.

Many workers use a very close series of alcohols in dehydrating and a similarly close series in replacing alcohol with the paraffin solvent, and then undo all their careful work by indiscriminately adding paraffin to the solvent.

A method of fixing paraffin ribbons to the side with certainty

Albumen fixative, which is almost universally used to fasten paraffin ribbons to the slide, has many excellences and a few disadvantages. Among the latter is the property of coagulating when subjected to moderate heat, and in consequence losing its adhesive quality. Because of this it is sometimes impossible to use sufficient heat to straighten refractory or much wrinkled ribbons, especially if paraffin melting at 58–60° is used. Again, it is almost impossible to fix sections of certain refractory plants to the slide, even if the ribbons are first straightened by floating on warm water and then transferred to an albumen coated slide and allowed to dry without heating. This is particularly true of sections of antheridial and archegonial heads of some mosses and of the strobili of *Selaginella*.

In anatomical work with seedlings and sporelings it is necessary to have an absolutely unbroken series, extending sometimes over many slides, in which the loss of a single section would destroy the value of the entire series.

The well known principle that most colloidal substances, when treated with a solution of some salt of chromium, exposed to light and dried, become insoluble in water, was utilized with complete success. The modern photographic processes, such as printing in pigmented gums and gelatin, photogravure, etc., are based on this property of bichromated colloids.

In the Hull Botanical Laboratory the writer and his students first tried Le Page's liquid glue thinned to the consistency of albumen fixative and made slightly yellow by dissolving a small quantity of potassium bichromate in the thinned glue. The slide was smeared with a thin coating of the bichromated glue and dried in the light. Later a solution of gum arabic was tried with even better results.

The present practice is to spread a few drops of a 1 per cent solution of gum arabic on the slide, taking care to see that every portion of the surface is covered, and flood the slide with water made slightly yellow by dissolving in it a few crystals of potassium bichromate. The ribbons are then straightened out on the slide by means of heat, the excess solution drained off, and the preparation put aside in the light to dry. A very short exposure to light is sufficient to render the gum insoluble in water. After the slides are thoroughly dry they are treated in the usual manner.

In heating the slide to straighten out the ribbons no special precaution, such as necessary with albumen fixative, need be taken, since gum arabic does not lose its adhesive power at temperatures below the melting point of the hardest paraffin ever used in imbedding. The paraffin in the ribbon may even be melted without lessening in the slightest the adhesive property of gum arabic.

When a large number of slides are to be made it is very convenient to mix the gum arabic and the potassium bichromate solutions and flood the slide with the mixture. The solutions should be mixed immediately before using, since the mixture does not keep.

Albumen fixative is much improved if, instead of water, the bichromate solution is used.

In the foregoing process the strength of the bichromate solution seems to be immaterial. If it is stronger than 1 per cent, crystals will appear when the preparation dries. These crystals do no harm, since they never appear in the sections, but they leave unsightly spots on the glass. In practice a 0.2 per cent bichromate solution will be entirely satisfactory. The writer does not make a solution of definite proportions, but adds enough potassium bichromate crystals to make the water pale yellow. A very small quantity of a salt of chromium is sufficient, in the presence of light, to render gum and gelatin insoluble in water.

Imbedding in gelatin

In preparing hard woods for sectioning it is the custom to soften in hydrofluoric acid and imbed in celloidin. Since this process involves dehydration, some refractory woods become unmanageable when sectioning is attempted.

In connection with the work of M. A. BRANNON on the extremely hard stems of plants which had been submerged by the rising waters of the Salton Sea and then exposed when the water receded, a method of imbedding in gelatin was devised by the writer and successfully used. Many of these stems were decorticated, some partially macerated, and all were excessively hard.

Gelatin is soaked in water until no more is taken up, the excess water drained off, and the gelatin liquefied by heat. Pieces of wood previously softened in water, or if necessary in hydrofluoric acid, are placed in the melted gelatin for some hours. Small blocks of hard wood to serve as supports in the microtome are also placed in the melted gelatin. The blocks to be sectioned are properly oriented in a gelatin matrix on the supporting blocks, cooled to set the gelatin, and plunged into strong formalin to harden the gelatin. In cutting the knife is flooded with water.

The advantages of this method are that no dehydration is necessary; that the process is very rapid; and that partly disintegrated tissues are held in place. In careful hands sections of hard woods can be cut as thin as is possible by the celloidin process.

Softening refractory material imbedded in paraffin

Plant material, especially if much starch is present, will not cut readily in paraffin. For complete infiltration with paraffin dehydration must be thorough, and a corresponding hardening of tissues results.

As is well known, paraffin is pervious to water. If imbedded material impossible to cut without fragmentation or tearing of the sections be stored in water for some weeks or months it will in most instances section readily. The effect of water on imbedded material will be most strikingly shown if an attempt is made to cut the gametophyte of some of the cycads at or just after fertilization of the egg immediately after imbedding, and again after the paraffin cakes have lain for some months in water. Dormant embryos of *Helianthus* which will not ribbon immediately after imbedding give unbroken ribbons after the paraffin block has been soaked for some weeks in water. The writer stores in water all paraffin containing hard material.

A method of cleaning cover glass

In attempting to clean cover glasses 50–60 mm. long by wiping with a cloth after they have been freed from the cleaning fluid, many are broken even with the most skilful and practiced handling. Also it is almost impossible to have them free from lint.

In the writer's practice cover glasses are placed in the usual cleaning fluid used for laboratory glassware, a mixture of sulphuric acid and potassium bichromate, rinsed under a tap to completely remove the acid, placed while wet in alcohol, and finally completely submerged in 95 per cent alcohol until wanted. To use, the cover glass is slowly withdrawn from the alcohol so that a minimum film of alcohol will remain on the glass, one end touched to a piece of absorbent paper free from dust to remove the drop of alcohol, touched to a flame, and when the alcohol has completely burned off placed while warm on the slide.

This method is very rapid and gives beautifully clean *cover glasses* with practically no breakage. If the cover glass is drawn from the alcohol so slowly that a very thin film remains, a small crack in the cover glass will not spread.

UNIVERSITY OF CHICAGO